(54) Title: HEPARIN DERIVATIVES

(57) Abstract

Low molecular weight heparin derivatives and salts thereof are prepared by treating a quaternary ammonium salt of a heparin, dissolved in a heterocyclic organic solvent or in an aprotic solvent, with an alkylating agent having from 6 to 30 carbon atoms at a temperature of about 20° to 60 °C for a prolonged period of time, treating the resultant reaction product at a temperature of about 5° to 120 °C with an inorganic or organic base, such as an alkaline hydroxide, in aqueous solution, and isolating the obtained heparin derivatives in their free form or as alkaline salts thereof. The heparin derivatives obtained according to the procedure of the invention constitute a mixture of mostly depolymerized products with a narrow range of molecular weight. These products are useful as anti-thrombotic agents.

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

Heparin derivatives

Background and Summary of the Invention

The present invention concerns new heparin
derivatives with pharmacological properties which are
5   modified with respect to those of the heparin
preparations currently available on the market and used
in anticoagulant therapies. The relationship between the
anti-thrombin activity and the activity on the Xa factor and the
platelet factor 4 (PF$_4$) of the new derivatives differs
10   from that of heparin. In other words, their considerable
affinity for PF$_4$ is accompanied by reduced activity on
the reaction between anti-thrombin III and thrombin,
catalyzed by heparin.

In parallel, the reaction between antithrombin III
15   and the Xa factor, catalyzed by heparin itself, is even more
sensitive to the new derivatives. Because of their
different ratio of activity from that of heparin, on PF$_4$
and on the reactions activated by thrombin and Xa factor,
the derivatives of the invention are able to prevent
20   thrombosis without the risk of hemorrhage which
accompanies the anti-thrombotic action of heparin. It
must be noted that hemorrhagic activity can be caused by
an excessive action of thrombin inhibition. The ability
of the new derivatives of the invention to bind PF$_4$, to
25   catalyze at low concentrations the reaction activated by the
Xa factor, and only at higher concentrations reactions
catalyzed by thrombin, make these compounds suitable as
drugs for the treatment of arterial and venous thrombosis.

The new heparin derivatives and their salts are
30   obtained by
(1)   prolonged treatment of an alkylating
(esterifying) agent derived from a hydrocarbon with more
than 6 carbon atoms, at room temperature or slightly
over, with a quaternary ammonium salt of heparin,
35   dissolved in a heterocyclic organic solvent chosen from
N-hydrocarbyl-pyrrolidine-2-one or

N-hydrocarbyl-piperidine-2-one, not substituted or
substituted by lower alkyl groups, and their derivatives
interrupted in the heterocyclic ring by a heteroatom or
hetero group chosen from -O-, -S- or -NH- or in a
5   concentrated solution of one such compound in an aprotic
solvent,

     (2)   treating the reaction product at a high
temperature with an organic or inorganic base in aqueous
solution,

10   (3)   isolating the heparin derivatives obtained in
free form or their alkaline salts, and, if desired,

     (4)   converting the resulting compounds into the
free form or into the alkaline   salts or as salts of
other metals or organic bases.

15   The new heparin derivatives, whose aforesaid
pharmacological properties are different from those of
heparin, also have a modified chemical structure and
different molecular weight; their chemical structure has
not yet been completely defined. It has however been
20   ascertained that they:

a)   constitute a mixture of mostly depolymerized
      products and therefore have a medium-to-low
      molecular weight compared   the
      starting products, which are rich in fragments
25   similar to one another and with molecular weights
      coming within the same narrow range, and with a
      minimum of fragments which deviate from this mean.

b)   Compared to their starting products, they have a
      notably diminished ratio between the iduronic-type
30   saccharide unit and the glucuronic-type unit.

Other modifications of the chemical structure, with
respect to heparin, which can be found in the new
derivatives, are in part also those which have already

been described for some heparin fragments obtained by
alkaline treatment of heparin esters, for example
desulfation and the presence of unsaturated monosic
units.

5   The identification of the new derivatives can be
carried out, apart from said chemical tests, by
spectroscopy, especially nuclear magnetic resonance
(NMR), which allows in particular to differentiate the
new derivatives from heparin fragments already described
10   in the literature.

The treatment of a quaternary ammonium salt of
heparin with an alkylating agent chosen from those
commonly used, that is, derived from a lower hydrocarbon,
such as a lower alkyl halide, for example ethyl bromide,
15   in a normally used solvent, such as dimethylsulfoxide or
dimethylformamide, leads to heparin esters, such as those
described in British patent No. 1501095. If these esters
undergo alkaline treatment, such as that included in the
second phase of the procedure of the present invention,
20   different heparin fragments are obtained, as can be
demonstrated by NMR analysis.

The new low-molecular-weight heparin derivative described in
the examples, constitutes a novel   product, which can
be characterized mainly on the basis of NMR spectroscopic
25   data at high frequency (400-500 MHz for the proton analysis;
100-125 MHz for C-13 analysis), utilizing traditional
one-dimensional spectres in FT (Fourier Transformation),
corroborated with the latest two-dimensional techniques
(H-H and C-H correlation), optionally resorting to more
30   recent methods (e.g. TOCSY = total correlation
spectroscopy), where necessary for the study of such
complex products.

From an overall examination of the results obtainable by the cited methods, it can be determined that:

1) The new "low-molecular-weight heparin" of the invention is a complex mixture of oligosaccharides, sulfated both at N and at O, reflecting the heterogeneity of the starting heparin. Its fundamental constituents IdoA and GlcnAc, differently sulfated, and secondary constituents (essentially GlcA) link together, giving rise to different sequential combinations.

2) With respect to the starting heparin, the product which is the object of the present invention shows a considerable decrease in the IdoA/GlcN ratio, this decrease being detectable by NMR. Accordingly, the signals of the anomeric sites of the typical IdoA sequence $(2SO_3)-GlcNSO_3$ $(6SO_3)$ (102 and 99.5 ppm for carbon; 5.18 and 5.32 ppm for the proton) prove to be drastically diminished. Such transformations cannot be detected in the known, low-molecular-weight heparins, or if they can, it is to a far lesser degree. Concomitantly, markedly intense signals appear of a new unsaturated monosaccharide unit (see point 4).

3) Another characteristic fact is the appearance of new signals (at 54.2 and 53.3 ppm for carbon, correlatable to protons at 3.73 and 3.68 ppm respectively) which is compatible, in the present context, only with the 2-position of a GlcN residue, with no SO_3H groups at the nitrogen). This characteristic, detectable by 2-D NMR, is practically absent in samples of known, low-molecular-weight heparins.

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4)   Typically, most of the aforesaid oligosaccharides
      terminate with an unsaturated monosaccharide unit,
      three-substituted in positions 1-2, as can be shown
      by NMR (signals at 147 and 172 ppm in C-13: doublet
      at 6 ppm for the proton). These signals are absent
      or very reduced in the other known,
      low-molecular-weight heparins.

      It must therefore be concluded that as early as the
first step of the procedure of the present invention,
what happens is not just a simple esterification, but a
more complex chemical reaction.

      It can therefore be assumed from these findings that
the heparin-like products of the present invention are
new and different from the heparin fragments already
described in the literature. For example, they are
different from the fragments described in European patent
No. 0 302 034, obtained by alkaline treatment of heparin
esters, these fragments being suitable for the formation
of complexes with copper ions having an angiogenic
action. In the same way, the heparin fragments described
in U.S. patent No. 4,440,926, obtained starting from
well-defined heparin esters by treatment with NaOH
(0.1-0.5 N) between 20° and 60°C, are not identical to
      the products of the present invention.

      The aforesaid qualities of the new heparin
derivatives of the present invention can be demonstrated
by the following experiments, performed on the product
described in the illustrative Examples and abridged to the
code name PE. In the experiments, unfractionated heparin
(UFH) and a low-molecular-weight heparin derivative (CY
216 = Fraxiparine®) are used as comparison products. Both
these products are commercially available for the
treatment of thrombosis.

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Brief Description of the Drawings

Figure 1 shows the anticoagulant activity _in vitro_
of PE compared to UFH and CY 216 in terms of thrombin
time (sec.). The data obtained are mean values of 7-10
replications for each test product.

Figure 2 shows the _ex vivo_ anticoagulant activity of
PE, compared to UFH and CY 216, according to the effect
on thrombin time after i.v. administration. The data are
mean values from experiments on 5 rabbits for each test
product.

Figure 3 illustrates the _ex vivo_ anticongulant
activity of PE, compared to UFH and CY 216, based on
anti-FXa activity (disappearance kinetics after s.c.
administration). The data are mean values from
experiments on 5 rabbits per test product.

Figure 4 shows the _ex vivo_ activity for PF_4 after
i.v. administration of PE, in comparison to UFH and CY
216.

1. ANTICOAGULANT ACTIVITY _IN VITRO_ ON RECONSTRUCTED
   HUMAN PLASMA

Materials and Methods

Test products (solubilization and concentrations)
The following products were tested:
- heparin derivative PE
- unfractionated heparin (UFH)
- low-molecular-weight heparin derivative (CY 216 -
  Fraxiparine®)
The test products were dissolved in sterile saline
and tested at concentrations ranging from 0.5 to
12.5 µg/ml.

Parameters

1. Thrombin time (as an indicator of anticoagulant
   activity)
2. Anti-FXa activity (as an indicator of anticoagulant
5 activity)

                          Results

1. Thrombin time
         From   Fig. 1 it is clear that:
10 - PE has anticoagulant activity starting at
     concentrations of 1.25 µg/ml
   - The anticoagulant activity of PE is considerably
     inferior to that of unfractionated heparin UFH
     (thrombin time increases at concentrations about
15   4-5 times greater than   UFH).
     No significant differences from CY 216 can be
     observed.

2. Anti-FXa activity
20   In Table 1, the preliminary data indicate
   that:
   - PE has anti-FXa activity starting from
     concentrations of 3 µg/ml
   - the anti-FXa activity is distinctly inferior to
25   that of heparin.
     It must also be noted that this activity is also
     inferior to that of CY 216; in this case, however,
     the difference is less evident than that observed
     in comparison to UFH.

30

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Table 1

ANTI-FXa ACTIVITY IN VITRO OF PE IN COMPARISON TO
UNFRACTIONATED HEPARIN AND CY 216

CONCENTRATIONS (μg/ml)	UFH	CY 216	PE
0.5	0.336	-	-
1	-	0.331	0.342
1.5	0.281	-	-
2.5	0.212	-	-
3	-	0.261	0.296
3.5	0.179	-	-
5	-	0.200	0.245
7	-	0.158	0.201

The data are means of 3 experiments per concentration,
one for each test product.

2. ANTICOAGULANT ACTIVITY EX VIVO ON RABBIT PLASMA
   AFTER I.V. AND S.C. ADMINISTRATION

   The anticoagulant activity of the new heparin
derivative PE was assessed by ex vivo methods on rabbit
plasma. By "ex vivo" is meant in vitro measurement of
blood withdrawn from an animal to which the chemical
substance has been administered. Examined in particular
were the following:
a) thrombin time, on arterial blood after acute bolus
   intravenous administration (i.v.) of the test
   products;
b) anti-FXa activity, on arterial blood after acute

subcutaneous administration (s.c.).
This method, being highly sensitive, is
indicative of the "bioavailability" of the test
products, since it allows the kinetics of the
5   disappearance of the test products to be
monitored after subcutaneous administration.
   The test products were dissolved in sterile saline
solution and administered acutely at 0.86 mg/kg by the
i.v. route and at 1-2 mg/kg by the s.c. route.
10

Test description

a) Thrombin time
   This test assesses thrombin time on samples of
arterial blood from rabbits at various time
15   intervals (2-5-10-20-30-40 minutes) after i.v.
administration of a bolus of test product.
b) Anti-FXa activity
   By measuring the anti-FXa activity (by a chromogenic
test which is sensitive at very low concentrations of
20   compound) it is possible to monitor the presence of the
test products in the circulation for a very long time.
Samples of arterial blood (from the central artery of the
ear) were taken at various intervals (1-2-3-4-5-6-7-8
hrs) after s.c. administration.
25

Results

a) Thrombin time
        From   Fig. 2 it is clear that:
- PE has a low anticoagulant activity at 0.86 mg/kg
30   i.v., confirming the in vitro data
- the anticoagulant activity of PE is distinctly
  inferior to that of UFH, but does not differ
  significantly from that of CY 216.

b) anti-FXa activity

Fig. 3 shows that:

- PE (after s.c. administration at 1 mg/kg) remains
  present in the circulation for longer than UFH,
5   administered at twice the dose, that is, at 2
  mg/kg s.c.

  The disappearance kinetics of the heparin
  derivative PE is therefore slower than that of
  UFH, but similar to that of CY 216.

10

3. EX VIVO AFFINITY FOR PLATELET FACTOR 4 (PF$_4$)

    By the experiments described hereafter, the affinity
of the new heparin derivative PE of the present invention
15 was assessed for human PF$_4$, i.e. the "binding properties"
of such products for PF$_4$.

    The ex vivo technique used monitored the kinetics of
the disappearance of PF$_4$ from the circulation in the
absence and in the presence of heparin.

20   It is well known that heparin or glycosaminoglycans
(GAGs), administered before PF$_4$, markedly enhance its
kinetics (G. Cella et al., "Human platelet factor 4 and
protamine sulphate interaction with glycosaminoglycans in
the rabbit", Eur. J. Clin. Invest., Vol. 17, pp. 548-554
25 (1987).

    Compounds able to bind PF$_4$ should therefore greatly
prolong its presence in the circulation. This effect is
proportional to the quantity of GAG injected and to its
affinity for PF$_4$.

30   The test products were dissolved in sterile saline
solution and administered at 0.86 mg/kg i.v.

Description of the test

Administration of the test products was acute (intravenous bolus), at a dose of 0.86 mg/kg. Samples of arterial blood were taken 2-3 mins later from the central
5 artery of the ear (baseline sample). 5 minutes after administration of the first bolus, a second bolus of purified human PF_4 was given (30 μg/kg).

Samples were taken 1.5-2.5-5-10-20-30 minutes after administration of PF_4. These were centrifuged, stored and
10 assessed by the RIA test (commercially available kit) for human PF_4.

Results

As reported in Fig. 4, the data obtained indicate
15 that:

- PE has affinity for platelet factor PF_4 and gradually disappears from the circulation reaching low values about 30 minutes after administration.
- The affinity of PE for PF_4 is much lower (about 60%)
20 than that of UFH and the kinetics of the disappearance of the complex with PF_4 is far more rapid than that of UFH, while it is similar to that of CY 216.

25 4. ANTITHROMBOTIC ACTIVITY _IN VIVO_ IN A MODEL OF ARTERIAL THROMBOSIS IN RABBIT

The experiment described hereafter assessed the antithrombotic activity _in vivo_ of the new heparin
30 derivative PE. The objective was to test the efficacy of the products in preventing the formation of an arterial thrombus in an acute model of arterial thrombosis in the rabbit carotid artery, following endothelial damage and reduction of the vascular

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diameter.

The test products were dissolved in sterile saline
and administered by the i.v. route at concentrations
5   ranging from 1.2 to 6 mg/kg.

Description of the model

The experiments were performed on rabbits
anesthetized with Nembutal (30 mg/kg i.v., bolus) and
10   tracheotomized for forced breathing. A tube was inserted
into the femoral vein and artery for the continuous
administration of anesthetic and drug. Systemic blood
pressure was monitored and the carotid was fixed with a
flowmeter and snare. When the system stabilized, stenosis
15   was performed. After suitable intervals (not less than 20
minutes), mechanical damage was induced with surgical
forceps and stenosis was performed above the damaged
vessel. The decrease in flow was monitored   until it
reached the value zero (0), corresponding   to occlusion of the
20   vessel. The controlateral carotid was prepared in the same
way. The test products were then infused i.v. for eight
minutes in all, with mechanical damage being performed
after two minutes of infusion. The flow was monitored
constantly until at least one hour after the end of
25   treatment. The animal was sacrificed   by an overdose of
anesthetic at the end of the experiment.

Results

As reported in Table 2, the data obtained indicate
30   that:
   - PE is effective in completely preventing the
      formation of an occlusive thrombus. The effect is
      evident at a dose of 6 mg/kg i.v.;
   - The antithrombotic efficacy of PE is inferior

(about 5x) to that of unfractionated heparin UFH
(which can be seen to inhibit the formation of
occlusive thrombi at a dose as low as 1.2 mg/kg); the
pharmacological efficacy of PE is similar to
that of CY 216.

Table 2

Antithrombotic activity of PE in comparison to
unfractionated heparin (UFH) and CY 216 in a model
of arterial stenosis in rabbit

Products	i.v. dose	No. occluded animals / No. treated animals
UFH	1.2 mg/kg	2/12
PE	6 mg/kg	2/12
	3 mg/kg	6/8
CY 216	6 mg/kg	2/12
	3 mg/kg	5/9

5. ANTITHROMBOTIC ACTIVITY IN A MODEL OF VENOUS THROMBOSIS _IN VIVO_

The experiment described hereafter was performed to assess
the antithrombotic efficacy _in vivo_ of PE in a
model of venous thrombosis in the rat.

The venous stasis technique was used, by occluding
the vena cava inferior, as this method causes the
formation of a mainly fibrinic thrombus due to the
variation in blood flow at the level of the bifurcation
of the vena cava with the left renal vein.

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The model under examination therefore proves to be
sensitive to the activity of anticoagulant drugs and in
particular to the antithrombotic potential of heparin
(after administration and stasis).

5   The test products were dissolved in sterile saline
and administered intravenously at doses ranging from 0.5
to 3 mg/kg i.v. (15 minutes before stasis induction).

Description of the test

10 The experiments were performed on male CD-COBS rats,
weighing 175-200 grams. The animals were anesthetised
with sodium pentobarbital (40 mg/kg i.p.) and their
undersides were disinfected with alcohol. A 4-cm incision
was made along the middle of the abdomen starting from

15 the ribs. The vena cava was isolated from the aorta to
the common iliac artery and tied with a cotton thread
immediately underneath the fork with the left renal vein.
The double occlusion was such as to exert constant and continuous
pressure for 5 seconds. The abdominal wall was then

20 closed. Two hours later the abdomen was reopened to verify
thrombus formation. After closing off the vena cava, in
correspondence to the common iliac arteries, and the
collateral circulation with a hemostat, the vascular
section below the occlusion was cut   longitudinally with a

25 needle. The thrombus was removed and immersed in distilled
water, blotted dry on paper and then placed in a dry box
for 24 hours to determine the dry residue
expressed in mg.

Results

The data relative to the weight (mg) of the thrombi (Table 3) and incidence (%) of the thrombi (Table 4) two hours after venous stasis, show that:

- PE has antithrombotic efficacy; its protective effect (pre-treatment) is significant at a dose of 1.5 mg/kg i.v.

- the efficacy of PE is inferior to that of unfractionated heparin UFH (which already has a significant effect at 0.5 mg/kg i.v.)

Table 3

Antithrombotic effect _in vivo_ of PE compared to UFH
in a venous stasis model: weight (mg) of the thrombi
obtained 2 hrs after venous stasis (effected 15
minutes after i.v. administration of the test
products)

		Doses (mg/kg i.v.)				
Products	0	0.5	1.0	1.8	2.0	3.0
	n = 15	n = 14	n = 24	n = 20	n = 14	n = 14
PE	2.2	1.4	1.5	0.8*	0.0**	0.0**
UFH	2.2	0.7*	0.5**	0.02**	–	–

$*$ $p < 0.05$; $**$ $p < 0.01$ Dunnett's test
n = number of animals

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Table 4

Antithrombotic effect _in vivo_ of PE compared to UFH in
a venous stasis model: incidence (%) of thrombi obtained
2 hours after venous stasis (effected 15 minutes after
i.v. administration of the test products)

Products	Doses (mg/kg i.v.)					
	0	0.5	1.0	1.5	2.0	3.0
	n = 15	n = 14	n = 24	n = 20	n = 14	n = 14
PE	93.3	85.7	62.5	60.0	0.0	0.0
UFH	93.3	57.1	50.0	14.3	–	–

n = number of animals

6. EFFECT ON BLEEDING TIME IN RAT

    The experiment described hereafter assessed the
effect of PE on bleeding time 15 minutes after
intravenous administration of the test products. The test
products were dissolved in sterile saline and
administered i.v. at concentrations ranging from 1 to 3
mg/kg.

Description of the test
    The experiments were performed on male, CD-COBS rats
(175-200 grams) in which the test substances were
administered acutely by the intravenous route. The
bleeding time (in seconds) was assessed 15 minutes later
by the standard template method of cutting the tail
(Dejana et al., "Bleeding time in rats: A comparison of
different experimental conditions", Thromb. Haemostat.,
Vol. 48, pp. 108-111 (1982)).
                    Results
    The data obtained (Table 5) show that:
- The effect of PE on "bleeding time" is
  significantly increased at a dose of 2.0 mg/kg
  i.v.
- the effect of PE is notably inferior to that
  obtained with unfractionated heparin UFH (in
  which the bleeding value already increases
  significantly at a dose of 1 mg/kg i.v.), but it
  is comparable to that of CY 216 (data not
  reported).
    It is interesting to note that in order to obtain
the same values for the bleeding time it is necessary to
administer a dose of PE which is at least 3 times
greater than that of UFH.

Table 5

The   effects of PE and UFH on bleeding time
(sec) 15 minutes after i.v. administration of the
test products

	Doses (mg/kg e.v.)				
Products	0	1.0	1.5	2.0	3.0
	n = 14	n = 14	n = 14	n = 14	n = 14
PE	130	146	147	250*	389**
UFH	130	413**	487**	-	-

* p < 0.05; ** p < 0.01 Dunnett's test
n = number of animals

7. IN VIVO TOLERANCE   AFTER I.V. AND S.C.
   ADMINISTRATION IN MOUSE

5   The present experiment assessed the maximum
tolerated dose of the compound PE after
administration by the intravenous and subcutaneous
route in the mouse.
        The test products were dissolved in sterile saline
and tested at concentrations ranging between 125
10   and 2000 mg/kg i.v. and s.c.

Description of the test
        The experiment was performed on male and female
CD-1 mice (C. River) (25-35 grams) and comprised two
15   steps.
     1) Screening. Groups of one male mouse and one
        female mouse were treated with one of the
        following doses: 125, 250, 500, 1000 and 2000
        mg/kg. Mortality within 7 days of treatment was
20   monitored.
     2) Groups of five male and five female mice were
        treated (for each compound and each
        administration route) with the highest
        tolerated dose found on screening. The animals
25   were observed for 14 days, during which
        mortality and the presence of altered behaviour and
        general toxicity symptoms were observed. In
        the case of mortality a lower dose was used,
        until the maximum tolerated dose was found.
30   Where possible, autopsy was performed on the
        deceased animals.

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Results

1. Intravenous tolerance dose

As reported in Tables 6 and 7, the maximum
tolerated dose of the test compounds proved to
be the following:

- PE: 1000 mg/kg i.v.
- unfractionated heparin UFH: 125 mg/kg i.v.
- CY 216: 1000 mg/kg i.v.

    It must be noted therefore that PE has greater tolerance
    dose   than unfractionated heparin UFH.
Autopsy of the animals which died after
treatment with unfractionated heparin UFH at 500
and 250 mg/kg, revealed no macroscopic findings
except, in some animals, the presence of
probable residues of internal hemorrhage in the
intestine.

2. Subcutaneous tolerance dose

The results obtained, as reported in Tables 8 and 9,
show that the maximum tolerated doses of the test
products are as follows:

- PE: 2000 mg/kg s.c.
- unfractionated heparin UFH: 125 mg/kg s.c.
- CY 216: 2000 mg/kg s.c.

    As with the intravenous route, therefore, the
tolerance dose of PE is far greater than that of
unfractionated heparin (UFH), while it is similar
to that of CY 216. It is however important to highlight
that:

- In all the animals (10/10) treated with CY 216 (2000
  mg/kg s.c.) a circular area of skin affected by
  necrosis was observed on the back, corresponding to
      the injection site, the size of which differed
  from animal to animal (maximum diameter about 1 cm).

Autopsy revealed necrosis to be limited to the skin;
it did not therefore affect the underlying muscle.

- No anomalies were observed in animals treated with
PE (2000 mg/kg s.c.).

5 - The animals which died after treatment with UFH (250
mg/kg) presented extensive bruising in the
subcutaneous dorsal region, where treatment had been
performed.

The subcutaneous tolerance   data therefore show a

10 difference between the compounds PE and CY 216.
Indeed, although the maximum tolerance doses are the
same, in the case of CY 216 a marked area of
necrosis of the skin is present at the injection
site, which indicates that CY 216 is tolerated to a

15 lesser degree locally than PE (which presented no
anomalies).

Table 6

Lethality of compounds PE, CY 216 and unfractionated
heparin UFH after a single intravenous administration
(screening)

Treatment (mg/kg i.v.)	Deceased animals/Treated animals	
	males	females
PE 2000	0/1	0/1
PE 1000	0/1	0/1
PE 500	0/1	0/1
PE 250	0/1	0/1
PE 125	0/1	0/1
CY216 2000	1/1	0/1
CY216 1000	0/1	0/1
CY216 500	0/1	0/1
Heparin UFH 2000	1/1	1/1
Heparin UFH 1000	1/1	1/1
Heparin UFH 500	0/1	0/1
Heparin UFH 250	0/1	0/1
Heparin UFH 125	0/1	0/1

Table 7

Lethality of compounds PE, CY 216 and unfractionated heparin
(UFH) after a single intravenous administration

Treatment	Deceased animals/Treated animals			Time of death				
(mg/kg i.v.)	males	females		days after treatment				
			immediate*	1	2	3	4
PE 2000	3/7	1/5	2	–	1	–	1
PE 1000	0/5	0/5	–	–	–	–	–
CY216 1000	0/5	0/5	–	–	–	–	–
Heparin 500	0/5	2/5	–	2	–	–	–
UFH 250	3/5	0/5	–	3	–	–	–
125	0/5	0/5	–	–	–	–	–

*Within minutes of treatment

Table 8

Lethality of the compounds PE, CY 216 and
unfractionated heparin UFH after a single
subcutaneous administration (screening)

Treatment (mg/kg s.c.)	Deceased animals/Treated animals	
	males	females
PE 2000	0/1	0/1
PE 1000	0/1	0/1
PE 500	0/1	0/1
PE 250	0/1	0/1
PE 125	0/1	0/1
CY216 2000	0/1	0/1
CY216 1000	0/1	0/1
CY216 500	0/1	0/1
Heparin UFH 2000	1/1	1/1
Heparin UFH 1000	1/1	0/1
Heparin UFH 500	1/1	0/1
Heparin UFH 250	0/1	0/1
Heparin UFH 125	0/1	0/1

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Table 9

Lethality of compounds PE, CY 216 and unfractionated heparin
(UFH) after a single subcutaneous administration

Treatment	Deceased animals/Treated animals		Time of death				
(mg/kg i.v.)	males	females	days after treatment				
			immediate	1	2	3	4
PE 2000	0/5	0/5	–	–	–	–	–
CY216 2000	0/5	0/5	–	–	–	–	–
Heparin 250	1/5	2/5	–	2	1	–	–
UFH 125	0/5	0/5	–	–	–	–	–

CONCLUSIONS

       The _in vitro_ and _in vivo_ results previously
described outline an interesting pharmacological profile
of the heparin derivative PE, setting it apart from
unfractionated heparin (UHF) and rendering its
pharmacological activities more valuable.

       The activity of PE differs slightly from that of CY
216, which is one of the best low-molecular-weight
heparins currently on sale in France, and shows in
particular better local tolerability after s.c.
administration in the mouse. Indeed, although the
maximum tolerated doses are the same, all animals treated
with CY 216 (2000 mg/kg s.c.) had   evident necrosis
of the skin at the injection site, while the skin of the
animals treated with PE was unaffected.

       In the case of pharmacological activity _in vitro_ and
in vivo, it is interesting to note that PE has:
- anticoagulant activity, far inferior to that of
  UFH;
- bioavailability, after s.c. administration, much
  greater than that of UFH, thereby allowing fewer
  daily administrations for the same pharmacological
  effect;
- lesser side effects, with regard to UFH, as shown
  by the reduced bleeding time and by the absence of
  skin necrosis at the injection site, with regard
  to CY 216 (the best low-molecular-weight heparin
  on sale in France) which caused a local
  intolerance   phenomena (necrosis) in all the
  animals treated ;
- antithrombotic activity _in vivo_, as shown in both
  the model of venous stasis in the rat and in the model of
  arterial thrombosis in the rabbit. The product is
  therefore efficacious in preventing these

pathologies at dosages of 1.5 and 6 mg/kg i.v.,
respectively, which do not induce any significant
variations in the side effects.
In particular, with regard to the venous antithrombotic
5   activity,   the derivative PE, at
a dose of 1.5 mg/kg i.v., does not induce any
alterations in bleeding time, unlike UFH.

The new, low-molecular-weight heparin derivatives
10   according to the present invention can therefore be used
in the place of unfractionated heparin in all its
indications, as an anticoagulant and thrombocyte
aggregation inhibitor. The dose is adapted to each
particular case, and is normally around 1-7 mg/kg per
15   day, by the intravenous route.
The heparins to be used in the previously described
procedure of the present invention can be of any type and
of various origin, for example heparins extracted from
the intestine of pigs, cattle, and sheep and from ox
20   heart, especially any one of the products which are
commercially available or described in the literature.
Such products have widely ranging molecular weights, for
example between 2,000 and 30,000 daltons, especially
unfractionated heparin UFH, and also low-molecular-weight
25   heparins obtained by fractionation of standard heparins
(2,000-10,000 daltons) and heparin fragments with a
molecular weight of 500-10,000 daltons obtained by
partial depolymerization of standard heparins by chemical
or enzymatic means. The quaternary ammonium salts to be
30   used as starting material for said procedure can be
prepared in the known way, for instance by ion exchange
of the heparin in aqueous solution as a sodium or
potassium salt with resins based on quaternary ammonium
salts, for example a salified sulfonic resin with a

quaternary ammonium base. The quaternary ammonium salt
can be obtained by freeze-drying the eluate. As
quaternary ammonium salts, tetraalkylammonium salts
derived from lower alkyls may be advantageously used,
5   particularly tetraalkylammonium salts with alkyl groups
having a maximum of 6 carbon atoms. Optionally,
alkyl-aryl-ammonium salts may also be used, for example
those having long-chained alkyl groups. Of the
tetraalkylammonium salts, tetrabutylammonium salts are
10   preferably used. The quaternary ammonium salts to be used
as starting substance for the procedure of the present
invention are those obtainable by ion exchange in the
aforesaid manner using alkaline salts, for example sodium
or potassium salts, of heparin of the type used
15   commercially, that is, neutral salts. They are reacted
with an excess of quaternary ammonium ions so as to
obtain also the corresponding neutral salts.
     Quaternary ammonium salts are soluble both in the
aforesaid heterocyclic solvents, and in aprotic organic
20   solvents, such as dimethylsulfoxide and
dimethylformamide. The reaction with the alkylating
agent, according to the first step of the procedure, can
be performed both in the aforesaid heterocyclic solvents,
or in a solution of the same in one of the aforesaid
25   aprotic organic solvents, preferably in a concentrated
solution. Of the aforesaid heterocyclic solvents, the
preferred ones are above all those which are not
substituted in the cyclic structure, that is,
N-alkyl-o-aryl-2-pyrrolidone, or their derivatives
30   containing a heterocyclic atom in the cyclic structure,
such as the corresponding derivatives of imidazoline,
piperazine or morpholine. The N-alkyl groups of
heterocyclic solvents are derived preferably from a lower
alkyl with a maximum of 6 carbon atoms, and the aryl

group is primarily a phenyl group, optionally substituted
by 1 to 3 lower alkyl groups, especially methyl groups.
Preferably, N-methyl-2-pyrrolidone is used.

5   An alkylating agent derived from a hydrocarbon
having from 6 to 30 carbon atoms, preferably from 8 to 18
carbon atoms, is a compound easily capable of yielding
the corresponding hydrocarbyl groups, such as a reagent
ester group of an acid with an alcohol with a
10   corresponding number of carbon atoms, which can be an
aliphatic or araliphatic alcohol having preferably a
maximum of 18 carbon atoms, for example a hexyl, heptyl,
octyl or nonyl alcohol. The reagent esters can be derived
from inorganic or organic acids, such as hydracids,
sulfuric or sulfurous acid, or alkyl- or aryl-sulfonic
15   acids, for example methane-sulfonic or p-toluenesulfonic
acid. The esters of the hydracids are in particular
chlorides, bromides and iodides. The reaction between the
quaternary ammonium salt of the aforesaid alkylating
agent is performed at room temperature (20°C.)or slightly
20   higher, for example at a temperature of from 30 - 35°C.
but not exceeding 60°C, and is maintained for a prolonged
period of time of about 5 to 20 hours, typically about 16
hours.

       The reaction product can be isolated and
25   subsequently submitted to the second step of the
procedure, that is, to alkaline treatment, or it can be
converted directly into the final product, performing the
alkaline treatment directly on the solution, optionally
concentrated, of the reaction product of the first step.
30   It is also possible to evaporate the solvent under bland
(mild) conditions after the first step of the procedure
and to perform alkaline treatment on the residue. It is
therefore possible to perform the procedure of the
invention as a "one-pot" process. The basic treatment of

the reaction product between the quaternary ammonium salt
of heparin with the alkylating agent is performed at a
temperature of from 5° to 120°C., suitably at a
temperature between 50°C and 120°C, preferably about
5   70°C, for about two hours. Alkaline hydrates are used,
such as NaOH or KOH, or other inorganic and/or organic
bases, preferably at a concentration of between 0.1 and
1M. The bases are used in aqueous or alcoholic solutions,
but other solvents can be present which are miscible with
10   water or with an aqueous-alcoholic mixture, such as, for
example, the residue solvents used in the first step of
the procedure in the case of the aforesaid "one-pot"
process.

          If it is desired to isolate the reaction product of
15   the first step of the procedure, it can be precipitated
by the addition of an organic polar solvent, preferably
an aliphatic alcohol such as methyl or ethyl alcohol, to
which a basic buffer has preferably been added, such as
especially a basic salt of a carboxylic acid, e.g., an
20   acetate or propionate of sodium or potassium. Other basic
buffers can be used, such as alkaline bicarbonates or
alkaline phosphates. The precipitated product is
preferably washed with the polar solvent used in the
precipitation, for example methanol. It is advisable to
25   purify the precipitated product further by
reprecipitating it once more or several times, that is,
by dissolving the product in water and precipitating it
with an alcohol.

          The reaction product, comprising the
30   low-molecular-weight heparin derivative and having the
previously described properties, can be released in free
form either from a metal salt or from an organic base in
the conventional way. The alkaline salt corresponding to
the alkaline hydrate used in the second step of the

procedure may be obtained by neutralizing the solution
with an acid, e.g., 2M hydrochloric acid, purifying the
solution, extracting it with an organic solvent which
cannot be mixed with water, for example methylene
5   chloride, and repeating this operation several times.
The salt is then dialyzed with distilled water and sodium
chloride and freeze-dried.

      The products of the invention are used mainly in the
form of their metal salts or organic base salts. If it is
10   desired to obtain metal salts other than alkaline salts
corresponding to the ions of the alkaline hydrates used
in the aforesaid alkaline treatment, it is possible to
use ion exchange methods by known techniques.
Alternatively, it is possible to isolate the heparin
15   product in its acid form, adding the alkaline salt
obtained with the calculated quantity of a diluted strong
acid, such as hydrochloric acid or sulfuric acid,
extracting the product with a suitable organic solvent,
isolating the heparin compound in free form and then
20   converting it into the desired salt.

      Of the metal or organic base salts which can be used
according to the present invention, of particular
importance are those which are therapeutically
acceptable, such as alkali and alkaline-earth metals, for
25   example sodium, potassium, ammonium, calcium, and
magnesium salts, or heavy metal salts, such as copper and
iron salts. The salts of organic bases can be derived
from primary, secondary or tertiary aliphatic, aromatic
or heterocyclic amines, such as methylamine, ethylamine,
30   propylamine, piperidine, morpholine, ephedrine,
furfurylaminecholine, ethylenediamine and aminoethanol.

      Salts which cannot be directly used in therapy,
which are also part of the invention, are for example
those which can be used for the purification of the new

heparin derivatives of the invention, such as some of the
heavy metal salts.

The invention also includes pharmaceutical
preparations containing as active substance a new
5   low-molecular-weight heparin derivative obtainable by the
procedure described herein, especially in the form of
their therapeutically acceptable salts. Such
preparations can be for parenteral use, for example for
subcutaneous or intravenous administration, or for
10   topical use, for example in the form of creams or
ointments, or suppositories, or nose sprays. They can
therefore be formulated as solutions of the active
compounds or as freeze-dried powders of the active
compounds to be mixed with one or more pharmaceutically
15   acceptable excipients or diluents which are convenient
for the aforesaid uses and with an osmolarity which is
compatible with physiological fluids.The invention
includes, besides the new low-molecular-weight heparin
derivatives, the aforesaid procedure for their
20   p⊥eparation. Included in the invention is also the
performance of the single steps of the preparation
procedure, that is, the reaction of a quaternary ammonium
salt of heparin with an alkylating agent as defined above
in one of said solvents and the alkaline treatment of the
25   reaction product.

The invention is illustrated in the following
Examples which, however, are not to be considered as
limiting.

1) FORMULATIONS FOR INJECTION

1.1

PE	mg	10	20	30	40
water for injection in ml		0.1	0.2	0.3	0.4

Package with ready-to-use syringe containing
sterile solution for subcutaneous administration.

1.2

PE	mg	150	300
other components:			
water for injection in ml		4	8

Package with vial containing sterile solution for
intravenous administration.

1.3

PE	mg	10	20	30	40
other components:					
sodium chloride	mg	0.3	0.6	0.9	1.2
water for injection in ml		0.1	0.2	0.3	0.4

Package with ready-to-use syringe containing
sterile solution for subcutaneous administration.

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1.4
    PE   mg   150   300
    other components:
    sodium chloride   mg   35   70
5   water for injection in ml   5   10
    Package with vial containing sterile solution for
    intravenous administration.

    1.5
10 PE   mg   150   300
    other components:
    sodium chloride   mg   35   70
    sodium metabisulfite   mg   5   10
    chlorbutanol (*)   mg   25   50
15 water for injection in ml   5   10
    Package with vial containing sterile solution for
    multidose intravenous administration.

    (*) in Example 1.5 the preservative chlorbutanol can
20 be substituted by:
    benzyl alcohol   mg   50   100
    or by:
    chlorocresol   mg   5   10

25

2) <u>FORMULATIONS FOR ORAL ADMINISTRATION</u>

2.1 (pill or capsule)

PE	mg	30	60	120
other components:				
phosphatidylcholine + phosphatidylserine (*)	mg	80	160	320
lactose	mg	50	100	200
microcrystalline cellulose	mg	10	20	40
talc	mg	3	6	12
colloidal silica	mg	3	6	12

(*) the ratios between the phosphatidylcholine and phosphatidylserine present in the formulations may vary

2.2 (pill or capsule)

PE	mg	30	60	120
other components:				
phosphatidylcholine + phosphatidylserine (*)	mg	80	160	320
cholesterol	mg	2	4	8
lactose	mg	50	100	200
microcrystalline cellulose	mg	10	20	40
talc	mg	3	6	12
colloidal silica	mg	3	6	12

(*) the ratios between the phosphatidylcholine and phosphatidylserine present in the formulations may vary.

2.3 (packet of powder)

PE	mg	60	120	180
other components:				
phosphatidylcholine +				
phosphatidylserine (*)	mg	250	500	750
cholesterol	mg	5	10	15
lactose	mg	100	200	300
flavoring and				
sweetener as required				
sucrose (**) to	mg	2000	4000	6000

(*) the ratios between the phosphatidylcholine and
phosphatidylserine present in the formulations may
vary

(**) sucrose may be substituted by fructose

2.4 (retard effect tablet)

PE	mg	30	60	120
other components:				
phosphatidylcholine +				
phosphatidylserine (*)	mg	80	160	320
lactose	mg	30	60	120
microcrystalline				
cellulose	mg	5	10	20
hydroxypropyl-				
methylcellulose	mg	10	20	40
talc	mg	3	6	12
colloidal silica	mg	3	6	12

(*) the ratios between the phosphatidylcholine and
phosphatidylserine present in the formulations may
vary

2.5 (gastroresistant tablet)

PE	mg	30	60	120
other components:				
phosphatidylcholine +				
phosphatidylserine (*)	mg	80	160	320
lactose	mg	50	100	200
microcrystalline				
cellulose	mg	10	20	40
talc	mg	30	60	120
colloidal silica	mg	3	6	12
methacrylic acid				
copolymer	mg	15	30	60

(*) the ratios between the phosphatidylcholine and
phosphatidylserine present in the formulations may
vary

2.6 (gastroresistant, retard effect tablet)

PE	mg	30	60	120
other components:				
phosphatidylcholine +				
phosphatidylserine (*)	mg	80	160	320
lactose	mg	50	100	200
microcrystalline				
cellulose	mg	10	20	40
hydroxypropyl-				
methylcellulose	mg	5	10	20
talc	mg	30	60	120
colloidal silica	mg	3	6	12
methacrylic acid				
copolymer	mg	15	30	60

(*) the ratios between the phosphatidylcholine and
phosphatidylserine present in the formulations may
vary

2.7 (solution to be administered orally as drops)

PE	mg	30	60	120
other components:				
citric acid	mg	7.5	15	30
purified water to	ml	0.25	0.5	1

2.8 (granules *)

PE	mg	30	60	120
other components:				
citric acid	mg	7.5	15	30
sucrose	mg	30	60	60
corn starch	mg	12.5	25	25
talc	mg	5	10	10
polyvinylpyrrolidone	mg	4	8	8

(*) 3 granules administered in a hard gelatin capsule

2.9 (gastroresistant granules *)

PE	mg	30	60	120
other components:				
citric acid	mg	7.5	15	30
sucrose	mg	30	60	60
corn starch	mg	12.5	25	25
talc	mg	5	10	10
polyvinylpyrrolidone	mg	4	8	8
methacrylic acid copolymer	mg	10	15	15

(*) 3 granules administered in a hard gelatin capsule

3. <u>NASAL OR PULMONARY FORMULATIONS</u>
3.1

PE fine white powder   mg   20   40

The active principle, as a fine white powder, is
5   contained in a gelatin capsule to be broken open
when ready for use and loaded into suitable
apparatus for inhalation.

3.2 (powder for inhalation)
10   PE fine white powder   mg   20   40
phosphatidylcholine +
phosphatidylserine *   mg   30   60

The active principle, as a fine white powder, is
15   contained in a gelatin capsule to be broken open
when ready for use and loaded into suitable
apparatus for inhalation.

(*) the ratios between the phosphatidylcholine and
20   phosphatidylserine present in the formulations may
vary

4. <u>FORMULATIONS FOR RECTAL ADMINISTRATION</u>
4.1
25   PE   mg   30   120
semisynthetic
glyceride   mg   1940   1880

4.2
30   PE   mg   30   120
phosphatidylserine +
phosphatidylcholine   mg   100   200
semisynthetic
glyceride   mg   1840   1680

In summary, the low molecular weight heparin
derivatives and salts thereof according to the present
invention are obtainable by treatment of a quaternary
ammonium salt of a heparin, dissolved in a heterocyclic
5   organic solvent chosen from the group formed by N-alkyl-
pyrrolidine-2-one, N-alkyl-piperidine-2-one,
unsubstituted or substituted by lower alkyl groups, and
their derivatives interrupted in the heterocyclic ring by
another heteroatom or heterogroup selected from -O-, -S-,
10   and -NH-, or in a concentrated solution of said compound
in an aprotic solvent, with an alkylating (etherifying)
agent derived from a hydrocarbon having from 6 to 30
carbon atoms, preferably from 8 to 18 carbon atoms, at a
temperature of from about 20° (room temperature) to 60°C.
15   for a prolonged period of time, e.g., about 5 to 20
hours. The resulting reaction product is then treated at
a temperature of from 5° to 120°C., preferably about
70°C., with an inorganic or organic base in an aqueous
solution. The product resulting from this alkaline
20   treatment is then isolated in free form or as an alkali
metal or alkaline earth metal salt thereof. Salts of
other metals or organic base salts may be obtained
therefrom by conversion from one to the other.
Preferably, in the second step of the procedure, the
25   reaction product is treated with an alkaline hydroxide in
aqueous solution. The resulting low molecular weight
heparin derivatives and salts thereof are useful
therapeutically, for example, as anti-thrombotic agents.

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CLAIMS

1.   A process for preparing low molecular weight
heparin derivatives and/or salts thereof which is
characterized by

(1)   treating a quaternary ammonium salt of a
heparin dissolved in a heterocyclic organic solvent
selected from N-alkyl-pyrrolidine-2-one, N-alkyl-
piperidine-2-one, unsubstituted or substituted by lower
alkyl groups, or derivatives thereof interrupted in the
heterocyclic ring by another heteroatom or heterogroup
selected from -O-, -S- or -NH-, or a concentrated
solution of said compound in an aprotic solvent, with an
alkylating agent derived from a hydrocarbon having from 6
to 30 carbon atoms, at room temperature or slightly
higher,

(2)   treating the resulting reaction product, at a
temperature of from 5° to 120°C., with an inorganic or
organic base in an aqueous solution, and

(3)   isolating the heparin derivatives obtained in
their free form or as alkali metal or alkaline earth
metal salts thereof.

2.   A process according to claim 1, which further
comprises converting said heparin derivatives or salts
obtained into salts of other metals or into organic base
salts.

3.   A process according to claim 1, which further
comprises converting the heparin derivatives obtained in
free form into pharmaceutically acceptable salts or
converting said salts into heparin derivatives in free
form.

4.   A process according to claim 1, wherein in step (2) said reaction product is treated with an alkaline hydroxide in aqueous solution.

5.   A process according to claim 1 or claim 4, wherein the product resulting from the reaction of the alkylating agent with the quaternary ammonium salt of heparin is isolated before being treated with the inorganic or organic base.

6.   A process according to claim 1 or claim 4, wherein quaternary ammonium salts of heparin having molecular weights varying from 2,000 to 30,000 daltons are used as a starting material.

7.   A process according to claim 1 or claim 4, wherein quaternary ammonium salts of unfractionated heparin are used as a starting material.

8.   A process according to claim 1 or claim 4, wherein quaternary ammonium salts of low molecular weight heparin, obtained by fractionation of heparin having a molecular weight of 2,000 to 10,000 daltons and heparin fragments having a molecular weight of 500 to 10,000 daltons obtained by partial chemical or enzymatic depolymerization of heparin, are used as a starting material.

9.   A process according to claim 1 or claim 4, wherein said quaternary ammonium salt is a tetraalkylammonium salt wherein the alkyl groups have from 1 to 6 carbon atoms.

10.   A process according to claim 1 or claim 4,
wherein said quaternary ammonium salt is the tetrabutyl
ammonium salt.

11.   A process according to claim 1 or claim 4,
wherein the N-alkyl group in said heterocyclic organic
solvent is a lower alkyl having from 1 to 6 carbon atoms.

12.   A process according to claim 1 or claim 4,
wherein said heterocyclic organic solvent is N-methyl-
pyrrolidone.

13.   A process according to claim 1 or claim 4,
wherein an ester of an acid with an aliphatic or
araliphatic alcohol having from 6 to 30 carbon atoms is
used as the alkylating agent.

14.   A process according to claim 13, wherein said
alkylating agent has from 8 to 18 carbon atoms.

15.   A process according to claim 14, wherein said
alkylating agent is an iodide, bromide or alkyl chloride,
or an ester of an alkyl- or arylsulfonic acid.

16.   A process according to claim 1 or claim 4,
wherein the alkylating agent is reacted with the
quaternary ammonium salt of heparin dissolved in said
heterocyclic organic solvent at a temperature of from
about 20° to 60°C.

17.   A process according to claim 1 or claim 4,
wherein the reaction product resulting from the reaction
of the alkylating agent with the quaternary ammonium salt

of heparin is isolated by precipitation by the addition
of a polar organic solvent.

18.   A process according to claim 17, wherein said
polar organic solvent is an aliphatic alcohol.

19.   A process according to claim 18, wherein a
basic buffer is added to said aliphatic alcohol.

20.   A process according to claim 19, wherein said
basic buffer is a basic salt of a carboxylic acid.

21.   A process according to claim 17, wherein the
precipitated product is reprecipitated several times with
the same organic solvent.

22.   A process according to claim 1, wherein in
step (2) said reaction product is treated with an
alkaline hydroxide or with another inorganic and/organic
base at a concentration of between 0.1 and 1M in aqueous
solution or alcoholic-aqueous solution at a temperature
of from 5° to 120°C.

23.   A process according to claim 22, wherein the
alkaline treatment step is performed on the isolated
reaction product resulting from step (1) with 0.1 - 1M
aqueous sodium hydroxide at a temperature of about 70°C.
for a sufficient time to obtain said low molecular weight
heparin derivatives and/or salts thereof.

24.   A process according to any of the preceding
claims, wherein the reaction product obtained from step
(2) is isolated by neutralization of the alkaline aqueous
solution, extraction with a water-immiscible organic

solvent, and dialysis with distilled water and sodium
chloride.

25.   A process for preparing lower molecular
weight heparin derivatives and/or salts thereof which is
characterized by
      (1)   treating the tetrabutylammonium salt of
heparin having a molecular weight of 15,000 daltons
dissolved in N-methyl-pyrrolidone with 1-iodooctane at
room temperature for about 16 hours,
      (2)   precipitating the reaction product by pouring
the resulting solution into methanol to which sodium
acetate has been added,
      (3)   purifying the resulting product several times
by reprecipitation from methanol to which sodium acetate
has been added,
      (4)   treating the dried product with 0.5 N aqueous
sodium hydroxide for about two hours at approximately
70°C.,
      (5)   neutralizing the resulting solution with 2M
hydrochloric acid,
      (6)   extracting the solution several times with
methylene chloride,
      (7)   dialyzing the solution with distilled water
and 0.1 M sodium chloride, and
      (8)   freeze-drying the obtained solution.

26.   Low molecular weight heparin derivatives and
salts thereof, prepared by the process as recited in any
of claims 1 - 25.

27.   Alkali metal or alkaline earth metal salts of
low molecular weight heparin derivatives prepared by the
process as recited in any of claims 1 - 25.

28.   A sodium, potassium or calcium salt of the heparin derivatives prepared according to any of claims 1 - 25.

29.   Salts of therapeutically acceptable bases of the heparin derivatives prepared according to any of claims 1 - 25.

30.   Sodium, potassium or calcium salt of the heparin derivatives prepared according to claim 29.

31.   A pharmaceutical composition comprising low molecular weight heparin derivatives according to claim 26 as an active ingredient and a pharmaceutically acceptable carrier, diluent or excipient.

32.   A pharmaceutical composition comprising an alkali metal or alkaline earth metal salt of low molecular weight heparin derivatives according to claim 26 as an active ingredient and a pharmaceutically acceptable carrier, diluent or excipient.

33.   The use of low molecular weight heparin derivatives according to claim 26 as a therapeutic agent.

34.   The use of alkali metal or alkaline earth metal salts of low molecular weight heparin derivatives according to claim 27 as a therapeutic agent.

35.   The use of low molecular weight heparin derivatives or salts thereof according to claim 26 for the treatment of thrombosis.

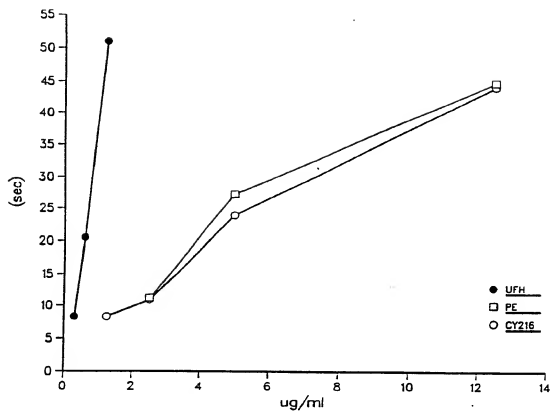36.   A pharmaceutical composition according to claims 31 and 32 for use in the treatment of thrombosis.
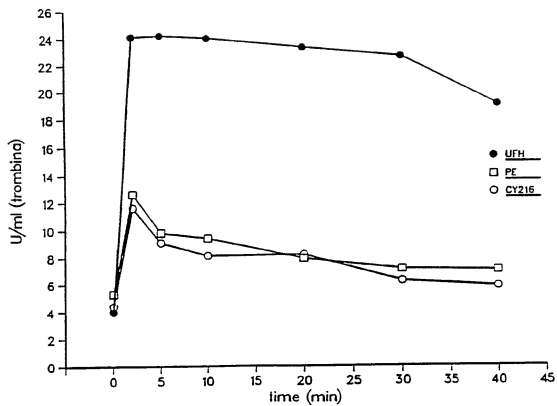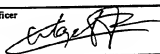
FIGURE 1

FIGURE 2

FIGURE 3
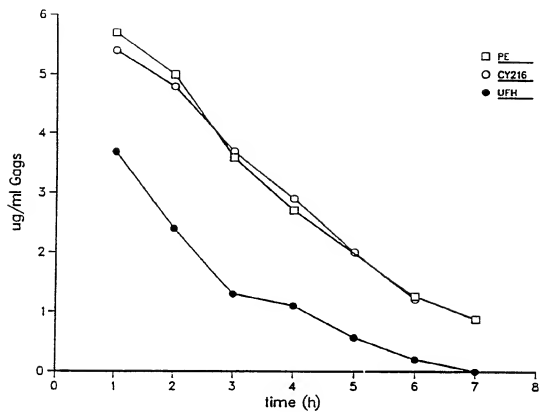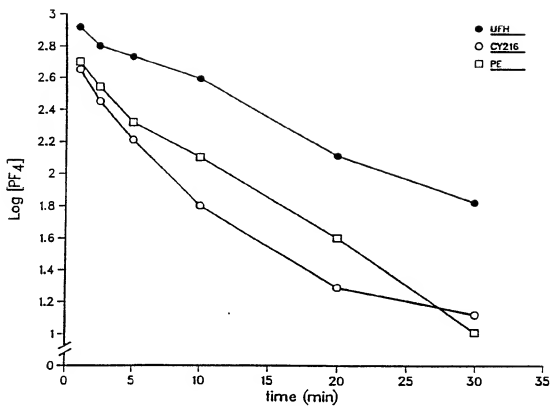
FIGURE 4

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C08B37/10;   A61K31/725

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C08B ; A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[°]	Citation of Document,[11] with Indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	EP,A,0 302 034 (KABIVITRUM AB) 1 February 1989 cited in the application see page 10; example 2 ---	1,4,5,7, 16-22
A	FR,A,2 482 603 (PHARMINDUSTRIE) 20 November 1981 see page 4, line 8 - page 6, line 5 & US,A,4 440 926 (J. MARDIGUIAN) 3 April 1984 cited in the application ---	33-35
A	EP,A,0 256 880 (MIAMI UNIVERSITY) 24 February 1988 see examples 1,7 ---	1

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28 FEBRUARY 1992	2 5. 03. 92

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	MAZET J.

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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